

# Photoporation Meets Stem Cell Therapies

## High-Efficiency Gene Delivery in Stem Cell Types



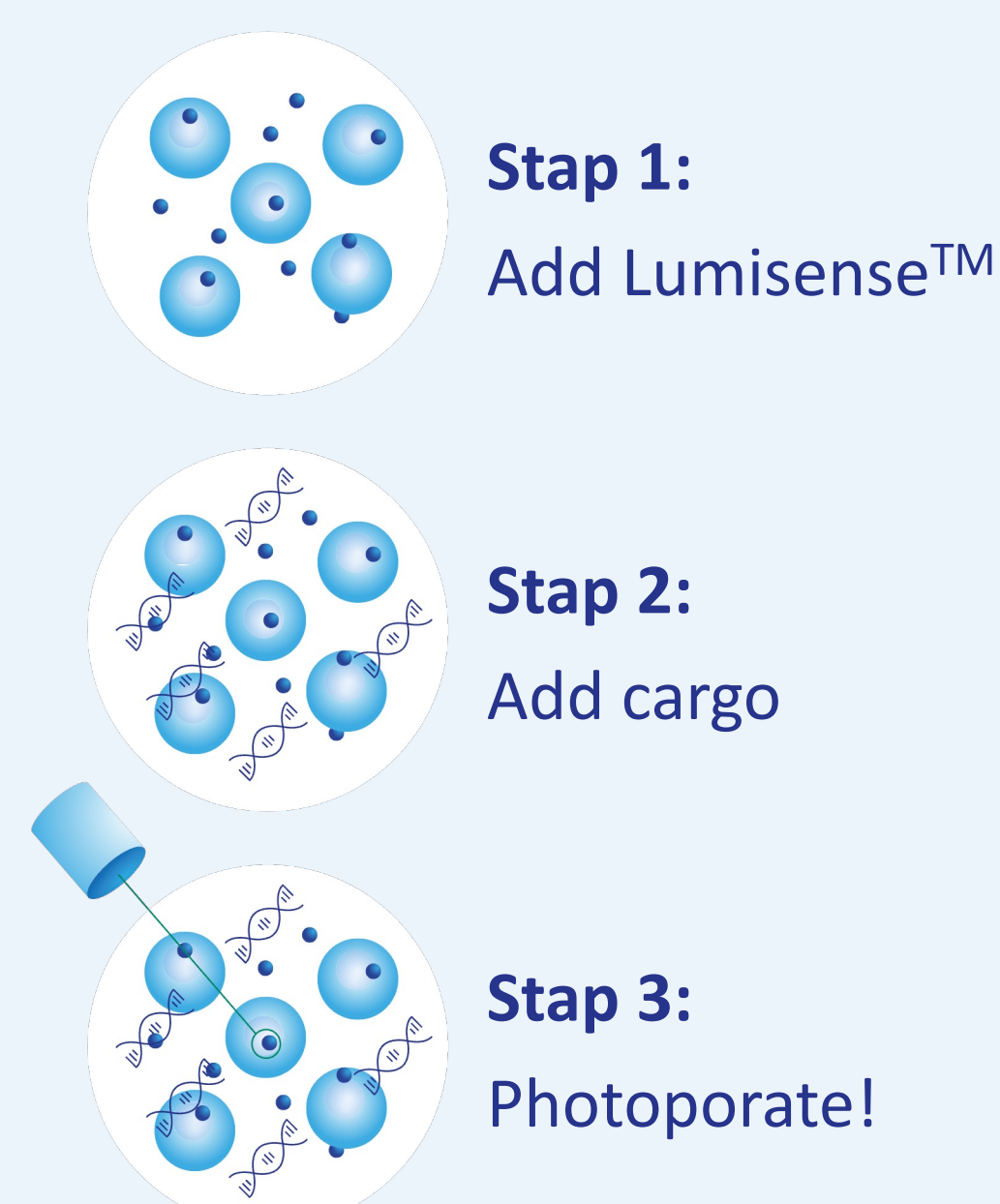
### Photoporation: a versatile transfection method

Challenge: **efficient delivery** of larger cargo in **hard-to-transfect cells**.

Trince's LumiPore™ intracellular delivery platform:

- **Laser** exposure combined with **photothermal nanoparticles**.
- **Transient permeabilization** of cellular plasma membrane.
- **High-throughput rate**, **high viability**, **proliferative capacity** and **functionality** of photoporated cells maintained.

#### The 3-step process



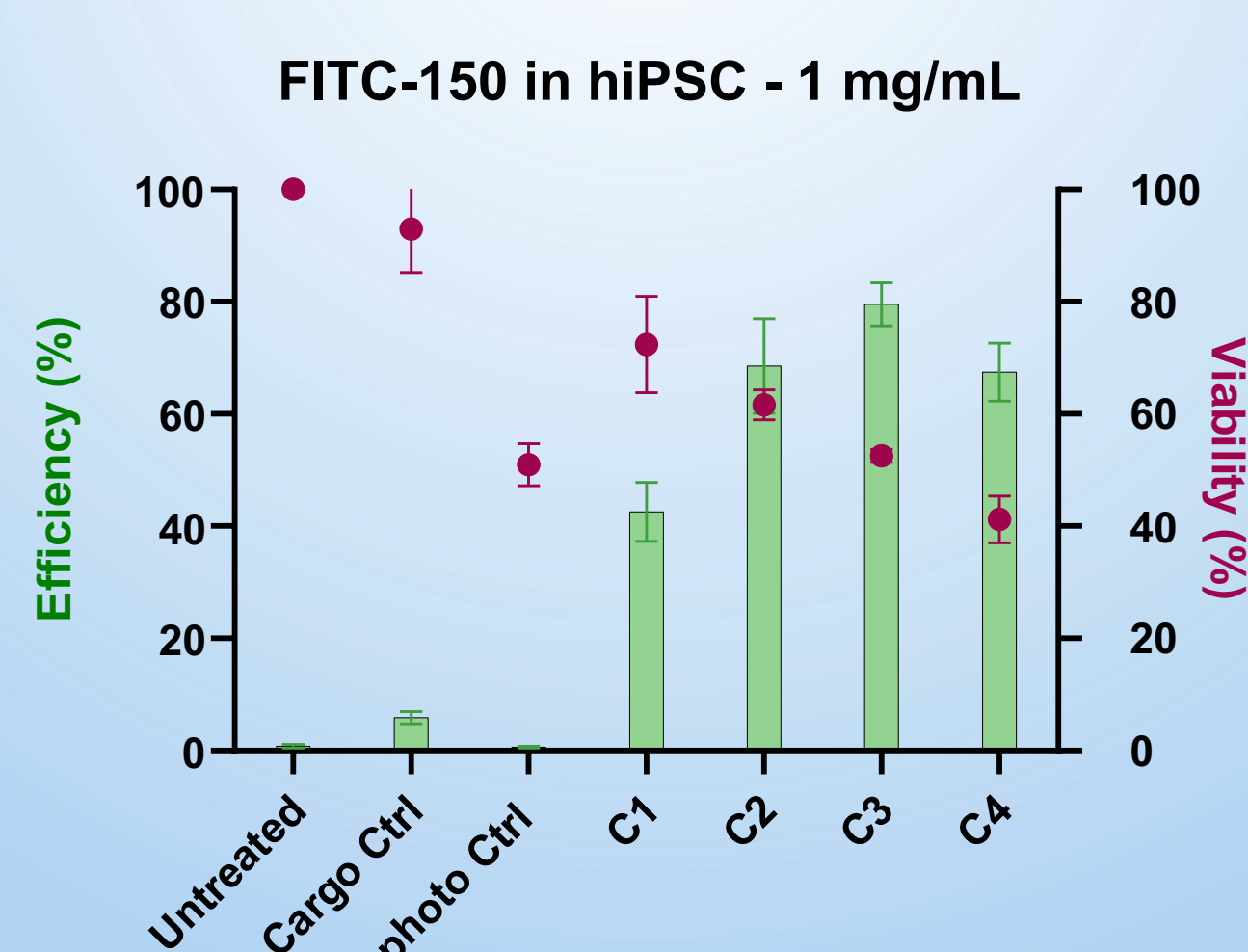
### LumiPore™ as the optimal transfection platform for stem cells and derivatives

**Stem cells**, including iPSCs, are valuable tools for **disease modelling**, **drug discovery**, and **regenerative medicine**. However, efficient intracellular delivery remains challenging due to their sensitivity to external perturbations.

**Photoporation** enables the intracellular delivery of nucleic acids, proteins, and small molecules through **gentle**, light-induced transient membrane permeabilization. By operating directly on adherent cells, it avoids disruptive detachment steps and preserves cell viability and function. The LumiPore™ platform is highly **flexible**, supporting efficient delivery across a broad range of cell-cargo combinations, including sensitive cell types such as iPSCs. Its **scalable** workflow can be readily adapted from low-throughput research studies to high-throughput screening applications, making it well suited for both fundamental and translational research.

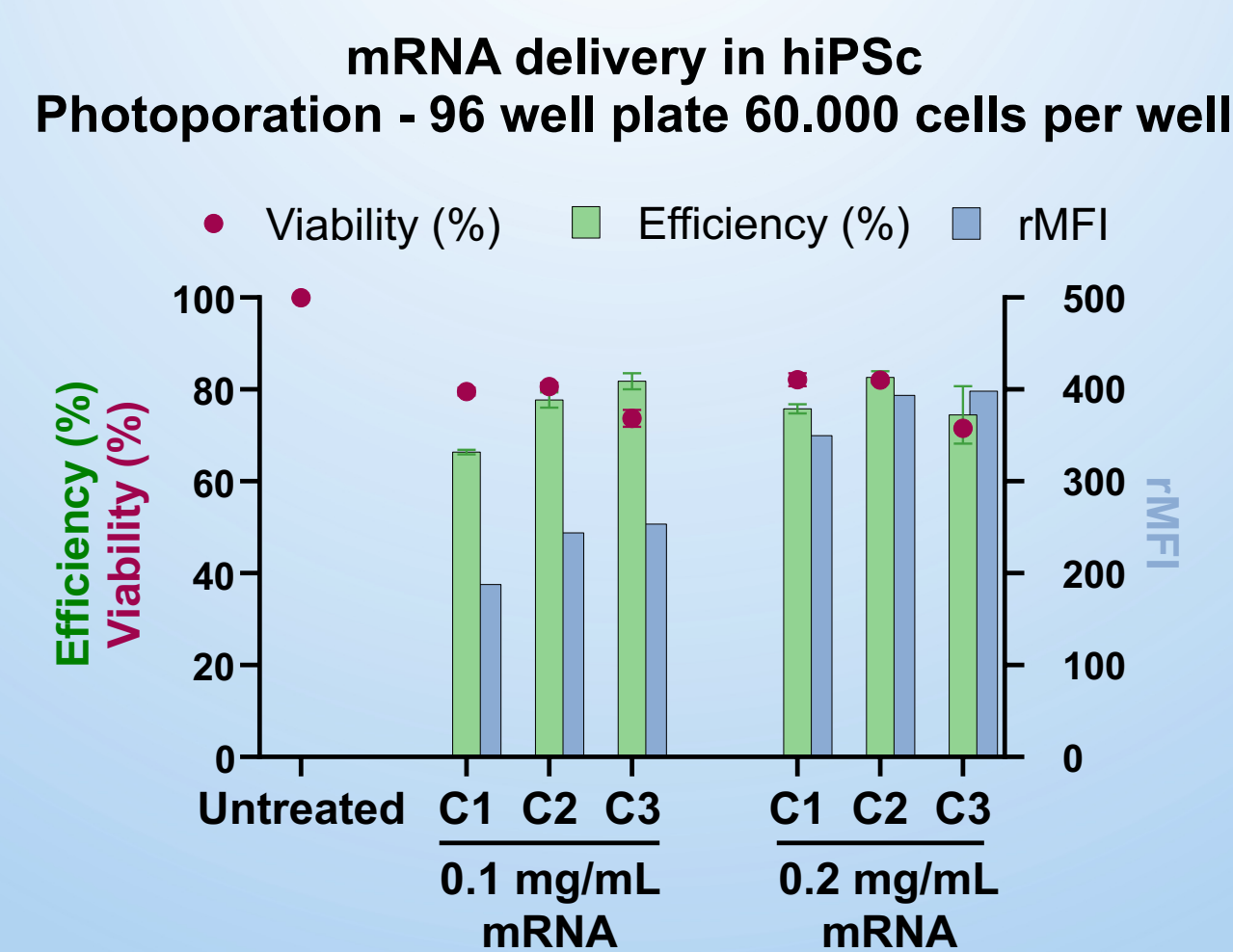
#### FD150 delivery for optimization in hiPSC

- Initial optimization on new cell lines (e.g. iPSC derivative) can be done in 4 hour process
- Range of Lumisense™ dilutions and range of laser powers indicate optimal conditions to work in
- First step before moving to functional cargo (ASO, siRNA, mRNA, RNPs)



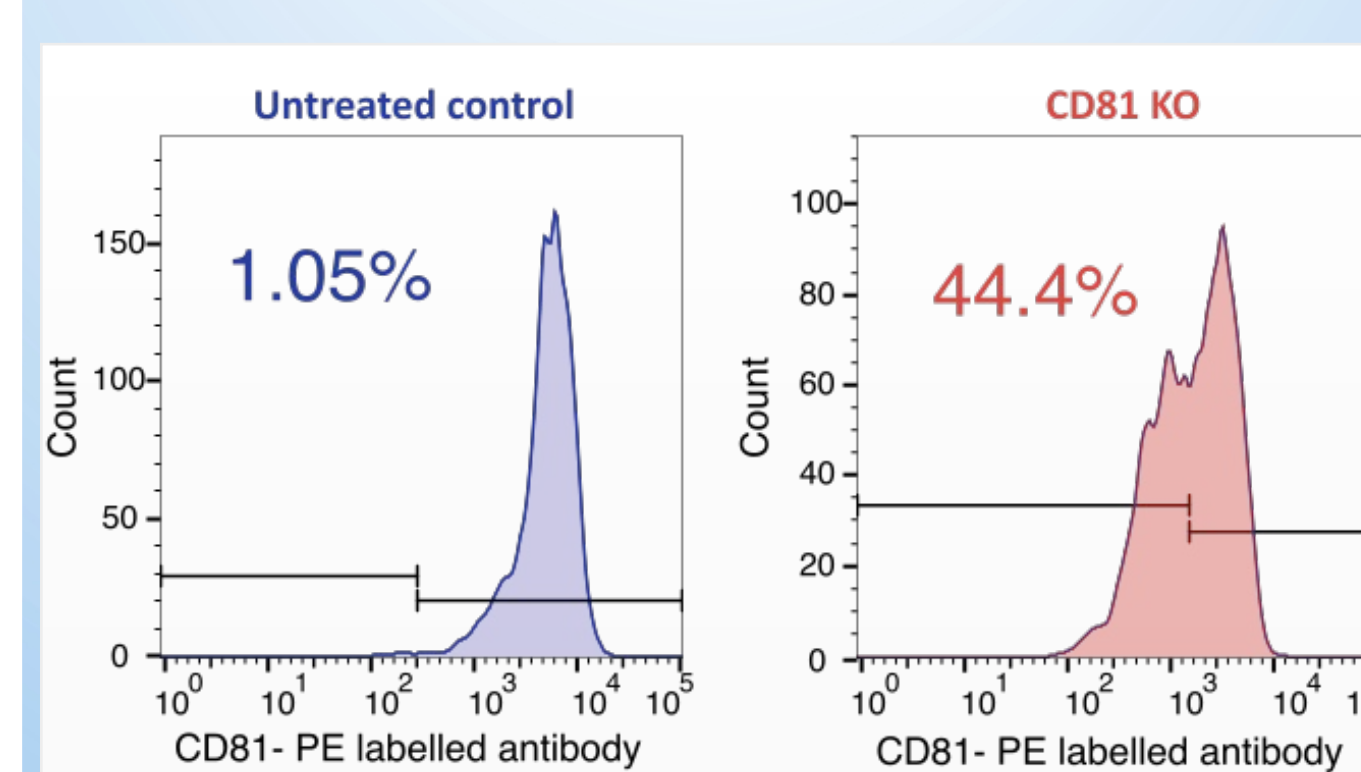
#### mRNA eGFP delivery in hiPSC

- Delivery of a 1 kb reporter mRNA
- Clear effect of cell density noticed on transfection efficiency (Efficiency %)
- Concentration of mRNA can be increased to increase expression per cell (rMFI)
- High viability is maintained while reaching > 80% transfection efficiency (Viability %)



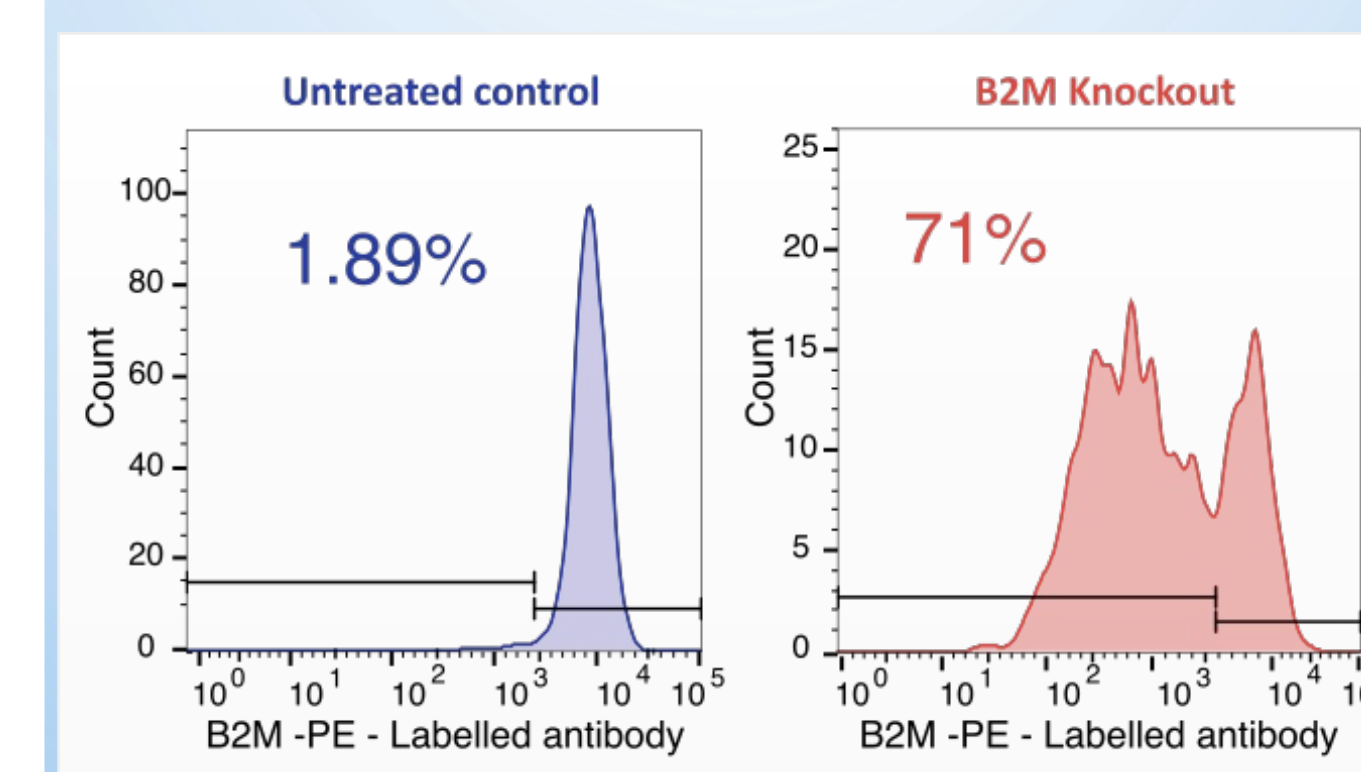
#### CD81 knockout in adherent hiPSC

- 50 000 cells per well in 96 well plate
- Delivery of precomplexed Cas9:sgRNA (1:2.5 ratio, 2  $\mu$ M:5  $\mu$ M)
- CD81 gene that codes for a surface expressed protein
- Readout using fluorescently labelled antibody
- > 40% KO with good viability



#### B2M knockout in primary BM-MSC

- BM-MSC maintained in adherent state
- Delivery of premade Cas9:sgRNA complex (1:2.5 ratio)
- B2M gene as target to lose MHC class 1 surface expression, therapeutic relevance for immune evasion
- > 70% KO can be achieved



### Conclusion

- Taken together, these results demonstrate the capability of photoporation and the Lumipore™ platform to achieve efficient intracellular delivery in iPSCs for both genome editing and transient gene expression applications. Efficient CRISPR-mediated knockout (> 44%) and high mRNA transfection (> 80%) were obtained while maintaining compatibility with adherent culture conditions. Combined with its scalable nature, photoporation therefore presents itself as a versatile and promising platform for stem cell engineering applications.

### LumiPore™ R&D device

- Benchtop intracellular delivery system
- Optimization per cell line possible
- Compatible with well-plates (6-1536), flasks and slides
- Full well plate in <10 min
- Ideal for high throughput and screening applications
- Special selectivity photoporation

